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Efficacy of a capsule preparation and ultraviolet-killed *Pasteurella haemolytica* A1 vaccine in goats

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Abstract

Weanling male Spanish goats ($n=38$) were randomly allotted to six treatment groups. Eight goats were positive controls; six goats were negative controls; six were given 2 transthoracic injections into the lung 21 d apart with 54 mg of purified Ph1 capsular polysaccharide (CPS) in agar beads; six had ultraviolet-killed Ph1 (1.4×10^9 CFU/ml) mixed with agar beads (Ph1-O); six had ultraviolet-killed Ph1 (8×10^9 CFU/ml) impregnated inside agar beads (Ph1-I); and six goats were given 2 subcutaneous injections 21 d apart with ultraviolet-killed Ph1 (8×10^9 CFU/ml) impregnated into agar beads (Ph1-I-SC). Thirteen d after the second injection all goats were challenge exposed to live Ph1 (1.93×10^8 CFU/ml) by transthoracic injection into the right lung, and 4 d later all goats were euthanatized and necropsied.

Mean volume of consolidated lung tissue was: for the negative control group, 150.83 cm^3; CPS group, 113.98 cm^3; positive control group, 0.06 cm^3; Ph1-O group, 11.68 cm^3; Ph1-I group, 11.24 cm^3; and Ph1-I-SC group, 33.47 cm^3. There was a significant difference ($P<0.04$) in lesion size among treatment groups. Mean values for treatment groups, positive control, Ph1-O, and Ph1-I were clustered together and all were considered immune. The Ph1-I-SC group was considered partially immune, and the NC and CPS groups were considered non-immune. All of the negative control goats died on d 35, five of the capsular group died by d 36. The transthoracicly injected, ultraviolet-irradiated whole cell bacterin, whether outside or inside the beads, was considered an excellent bacterin which induced solid immunity to a severe challenge exposure.

Keywords: BRD model; Shipping fever; Goat; *Pasteurella haemolytica*; Vaccine

1. Introduction

Pasteurella haemolytica (Ph) is frequently found in the upper respiratory tract of ruminant animals. These organisms can induce pneumonia in goats (Fodor et al., 1989; Hordagoda et al., 1981; Midwinter et al., 1986), sheep (Frank, 1982; Younan et al., 1988), and cattle (Lillie, 1974; Frank and Smith, 1983). Two biotypes (A and T) and 12 serovars are generally recognized (Biberstein, 1978; Frank and Wessman, 1978), although other investigators report 15 serovars (Fraser et al., 1982). Some strains are untypeable when standard typing serums are used (Frank, 1980).

Goat pasteurellosis appears to be a grossly underreported disease. Most goat pasteurellosis is in tropical countries perhaps due to their larger goat populations (Fodor et al., 1984). Little European literature on goat pasteurellosis is available, however, Fodor et al.

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(1989), reported that 28 isolates of Ph2 were recovered from pneumonic lungs from goats of all ages from two farms in Hungary. State Veterinary diagnostic laboratories in the USA do not routinely serotype *Pasteurella haemolytica* recovered from any ruminant species. Rodriguez et al. (1991) reported the isolation of *Pasteurella haemolytica* (serotypes were not determined) from 20 pneumonic goats from 0 to 120 d of age in Cuba. A report from Texas (Scanlan et al., 1993) described a naturally occurring outbreak of *Pasteurella haemolytica* A2 in market-stressed goats.

Pasteurella haemolytica A1 (Ph1) is the predominant pathogen in acute bovine respiratory disease complex (BRDC). This disease occurs most often in feeder and stocker calves following marketing stress and shipment by truck from the farm of origin to feedlots or stocker operations (Frank and Smith, 1983; Purdy et al., 1986).

The goat is an excellent model in which to test the safety and efficacy of Ph1 subunit vaccines and bacterins (Purdy et al., 1990, 1993; Purdy and Foster, 1991). The Ph1 immunity induced by vaccines in goats or calves can be quantified by measuring the volume of consolidated lung tissue 4 d after challenge. If immunity is induced by the vaccine, the volume of the consolidated lung tissue, post-challenge exposure, will be larger in a negative control group than in immune groups. The best immunity that one can expect from a Ph1 bacterin or subunit vaccine should compare favorably with that induced in a positive control group in which a live Ph1 vaccine was used (Purdy et al., 1990; 1993).

The purpose of this study was to compare immunity induced in goats by a Ph1 capsule antigen (CPS), ultraviolet-killed whole cell Ph1 and live Ph1. The capsule is thought to be a protective antigen in calves (Confer et al., 1985) and we showed it to produce partial immunity in goats (Purdy et al., 1993). Killing Ph1 with ultraviolet irradiation does not change the protein or carbohydrate antigens, however it does kill the bacteria by interfering with DNA replication (Lo and MacDonald, 1991).

2. Materials and methods

Fifty weanling, male Spanish goats were purchased from one herd located near Brady, TX. These goats had no history of respiratory disease or of being vaccinated with *Pasteurella* products. They were transported 644 km by truck to the United States Department of Agriculture, Agriculture Research Service, Research Laboratory located in Bushland, TX. On arrival the goats were treated for internal parasites (Ivomec-MSD AGVET, Merck & Co. Inc., Rahway, NJ) and coccidia (Amprolium, MSD AGVET, Merck & Co. Inc., Rahway, NJ), and conditioned to their new environment for 3 wk. They were housed in a covered, three-sided barn and were maintained on a pelleted sheep/goat ration (0.45 kg/goat/d), alfalfa hay ad lib and fresh water.

Preparation of Ph1 subunit capsule antigens

The Ph1 capsular polysaccharide (CPS) was purified (Purdy et al., 1993; Lowry et al., 1951; Kabat and Mayer, 1961; Osborn, 1963). Agar beads were impregnated with CPS, ultraviolet-killed Ph1 bacterin or live Ph1 (Cash et al., 1983). One part antigen was mixed with 9 parts of molten agar (46°C), poured into warmed (46°C) heavy mineral oil, immediately placed on ice, and vigorously stirred until agar beads formed. Beads were separated from the oil by mixing in a 0.5% solution of deoxycholic acid, followed by centrifugation at $432 \times g$. After supernatant was removed, beads were washed three times in phosphate-buffered saline (PBS). In the bacterin group (Ph1-O), the ultraviolet-killed Ph1 were mixed with agar beads so that the Ph1 was not incorporated inside the beads. This was done to determine whether similar results could be obtained without placing the bacteria inside the beads as others have reported (Nacucchio et al., 1984).

Bacterium

Pasteurella haemolytica A1 was isolated from a pneumonic calf and identified by colony morphology, Gram's stain, biochemical reactions, and by use of a specific serotyping antiserum (Frank and Wessman, 1978). The same bacterial stock was used to prepare the capsuler antigen.

Live vaccine, challenge inoculation and bacterin preparations

Live Ph1 vaccine and challenge-inoculum cultures (Purdy et al., 1990) were routinely grown on nutrient agar plus 5% bovine (citrate) blood for 16 h at 37°C in 5% CO_2. Cultures were harvested in PBS solution

(0.10 M, pH 7.2) and the bacterial concentration was determined by surface colony counts on the same medium.

The ultraviolet-irradiated cultures were grown on nutrient agar plus 5% goat blood at 37°C in a 5% CO_2 atmosphere for 10 h and harvested in 0.15 M saline solution (pH 6.6). The bacterial suspension was dispensed into uncovered petri plates to a depth of 5 mm and irradiated (Spectroline model TR-312 A Ultraviolet transilluminator, Spectronics Corp., Westburry, NY) at 315 nm for 60 min inside a vertical laminar flow biological hood. After irradiation, a sterile water rinse was added to the bacterial suspension. The ultraviolet-irradiated bacterial suspension was repeatedly cultured to insure complete killing.

Experimental design

Goats were randomly allotted to six treatment groups: (1) positive control (PC, $n=8$), live Ph1 impregnated inside agar beads; (2) negative controls (NC, $n=6$), agar beads only; (3) capsule (CPS, $n=6$), antigen impregnated inside agar beads; (4) ultraviolet-killed bacterin (Ph1-0, $n=6$), whole cells mixed with agar beads; (5) ultraviolet-killed bacterin (Ph1-I, $n=6$), whole cells impregnated inside agar beads; and (6) ultraviolet-killed bacterin (Ph1-I-SC, $n=6$), whole cells impregnated into agar beads and injected subcutaneously into the left thigh. The goats of the first five groups were each injected transthoracicly into the left posterior lung lobe with the appropriate antigen, bacterin or live vaccine-agar bead preparation.

PC goats were held in a separate barn to prevent possible Ph1 cross-contamination to other goat groups. Goats in groups 1 through 4 were tranquilized (100 mg thylisobutrazine HCL, IV., Diquel, Jensen Salsbury Laboratories, Division of Burroughs Wellcome Co., Kansas City, MO) 15 min before injection into the lung (Purdy et al., 1990).

Concentrations and doses of the antigen, bacterins, or live Ph1 vaccine given on d 0 were: (1) PC, 2.28×10^6 Ph1 CFU/ml, 1 ml/goat; (2) NC, agar beads only suspended in physiologic saline solution, 1 ml/goat; (3) CPS, 54 mg capsule antigen impregnated inside agar beads, 1 ml/goat; (4) Ph1-O, 1.4×10^9 ultraviolet-killed Ph1 CFU/ml, 1 ml/goat; (5) Ph1-I and (6) Ph1-I-SC, 8×10^9 ultraviolet-killed Ph1 CFU/ml, 1 ml/goat. The low dose of live Ph1 was selected to prevent severe pneumonia from developing. The ultraviolet-killed Ph1 vaccine dose was selected in order to have sufficient antigen mass to induce immunity. The CPS concentration and ultraviolet-killed Ph1 bacterin dose was the same on d 21. On d 21 the positive control goats were each given 1.25×10^6 Ph1 CFU/ml/goat.

All goats were challenge exposed on d 34 by transthoracic injection of live Ph1 (1 ml dose, 1.93×10^8 CFU) into the right posterior lung lobe. On d 38 they were euthanatized with an overdose of a barbiturate anesthetic and immediately exsanguinated (Purdy et al., 1990). Necropsies were preformed, and lungs were examined for Ph1-induced lesions. Consolidated lesions were measured (length × width × thickness) with calipers. Immune protection was evaluated on the basis of the volume of consolidated lung tissue.

Specimen collection

Blood samples were collected via jugular venipuncture on d 0, 7, 14, 21, 28, 34, and 38. A heparinized blood sample (3 ml) was used for total white blood cell (WBC) counts, packed cell volume (PCV), and WBC differential counts. Sera were collected from 25 ml blood samples. The blood was allowed to clot at ambient temperature for approx. 20 min, placed on ice for 1 h, and centrifuged at 4°C. Each serum was decanted into three cold glass vials and immediately frozen at −80°C. These serum samples were used to determine complement activity, Ph1 IHA antibody, and anticytotoxin neutralization titers.

Nasal turbinate mucus specimens were collected with swabs on d 0 to 3, 6, 7, 10, 14, 21, and 34 to 38. The specimens were stored at −85°C. After thawing, swabs were subjected to bacteriologic culture on 5% bovine blood agar and incubated at 37°C in a 5% CO_2 atmosphere. *Pasteurella haemolytica* isolates were identified by appearance of colony morphology, hemolysis, Gram's stain, and specific serotyping.

At necropsy, bacterial specimens were obtained from the left lung for culture by aseptically inserting a sterile cotton swab through an incision at the challenge injection site. Swab specimens were also taken from the right lung, and trachea. After the swab had absorbed approx. 0.1 ml of tissue fluid from the lung, it was removed and expressed into a tube. Serial 10-fold dilutions were created. A 0.1 ml sample of each dilution was spread onto a blood agar plate, incubated at 37°C

for 16 h in a 5% CO_2 atmosphere, and Ph1 colonies were counted.

Clinical observations

Goats were observed twice a day for adverse clinical signs throughout the experiment. Physical examinations were performed and rectal temperatures were recorded for all goats on d 0 to 3, 6 to 10, 14, 20 to 23, and 34 to 38. Goat body weights were recorded on d −21, 0, 7, 21, and 34. Goats were always treated in accordance with the Consortium Guide (1988).

Serum assays

Anticytotoxin antibodies were determined by a modification of the method of Chang et al. (1987) (CT neutralization). The assay used bovine lymphoma cells (BL3) as target cells and was performed in 96-well microtiter plates. Stock cytotoxin was titered prior to each use. One unit of toxin was defined as the quantity which caused complete lysis of 1×10^6 BL3 cells. Sera were diluted in L15 media (GIBCO) by doubling dilutions, then toxin was added 1:1 (v/v). Sera (100 μl)/toxin (100 μl) mixtures were incubated at room temperature for 10 min. Approx. 15,000 BL3 cells (100 μl) suspended in L15 media were then added to each well. Plates were incubated for 1 h at 37°C in a 5% CO_2 atmosphere and examined by microscopy for lysis of BL3 cells. The anticytotoxin titer was determined as the last dilution which gave >90% protection. Two control sera were used in each microliter plate (positive control – 1:2048 anticytotoxin titer and negative control fetal calf serum – no anticytotoxin activity). All serum samples from each animal were tested on the same day.

A standard serum classical hemolytic complement assay (Renshaw et al., 1980) was conducted and reported in mean CH50 units/ml of serum. All samples collected on the evaluation days from each goat were assayed on the same day. A positive bovine serum control (stored at −85°C) was included with each assay to determine daily test variation.

An IHA assay (Frank and Smith, 1983) was used to determine Ph1 serum titers which were reported in geometric means for the respective groups. All serum samples from each goat were assayed on the same day with a laboratory serum control.

Statistical analysis

Data were analyzed by analysis of variance using the general linear models procedure of SAS (1988). Variables included in the model statement were time, treatment, and time × treatment interaction. Differences among treatments and sampling days were compared by Duncan's multiple-range test if a significant *F*-test was obtained. Differences were considered statistically significant at $P < 0.05$. Anticytotoxin antibody titers and IHA antibody titers are reported as geometric means.

3. Results

Antigen characterization

There was no detectable protein or nucleic acid in the CPS preparation. There was also no detectable 2-keto-3-deoxyoctonate indicating no contaminating lipopolysaccharide (LPS). Three mg of Ph1 CPS gave only one homogenous peak (absorbance at 206 nm), which eluted from the Sepharose 2B column in the void volume, indicating a very high molecular weight (Fig. 1). Silver stained gels of the purified CPS also indicated material with a very high molecular weight which did not contain any LPS (Purdy et al., 1993).

Mortality after challenge exposure prior to termination of the experiment

Three goats in the PC group died 2 d after the first injection from a bilateral pneumonic pasteurellosis. Six NC goats and three CPS goats died on d 35, and two

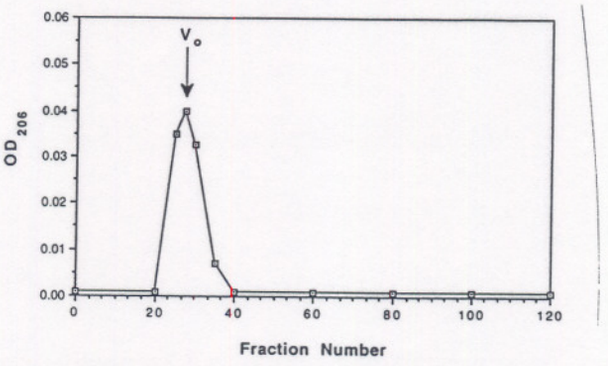

Fig. 1. Elution profile of the purified capsule carbohydrate of *Pasteurella haemolytica* A1 on Sepharose 2B employing 0.01 mM Tris-HCl buffer, pH 8.0. The eluate was monitored continuously for capsule carbohydrate by absorbance (OD) at 206 nm. V_o = void volume of the column.

CPS and one Ph1-I-SC goats died on d 36. All deaths were due to Ph1 pneumonia induced by the challenge exposure.

Rectal temperature recordings

There were significant differences ($P<0.006$) in rectal temperature among treatment groups on d 3, 7, 8, 9, 24, and 34 (Fig. 2). Body temperature increased within 24 h after the first live Ph1 injection of the lungs (PC group) and, in some cases, it lasted for a few days. After challenge exposure, the greatest increase in body temperature occurred in the Ph1-O, Ph1-I, and Ph1-I-SC groups and subnormal temperatures occurred in the NC and CPS groups.

Body weight

Mean BW of the goats on d −21 was 10.43 kg (range 9.8 to 11.3 kg). There were no significant differences in weight gain among treatment groups. The mean BW of the goats on challenge exposure d 34 was 16.2 kg (range: 10.1 to 20.6 kg).

Total white blood cell counts

There were significant differences in total WBC counts among treatment groups (Fig. 3) on d 7 ($P<0.045$) due to a mean increase of 7200 cells in the PC group. The range of WBC counts on d 7 in all other groups was 11,858 to 15,933. No significant differences in total WBC counts occurred among days in any treatment group.

Serum anticytotoxin antibodies

Passive antibody decreased from d −21 to d 0 in all groups (Fig. 4). Only the PC group had an increase in active anticytotoxin antibody after two vaccinations with live Ph1. The mean titer of the PC group was highest on d 34, 13 d after the second vaccination.

Serum hemolytic complement activity

There were significant differences in serum complement activity among treatment groups (Fig. 5) on day 21 ($P<0.05$). The average serum complement activity of all treatment groups over all sampling days was 78 CH50 units/ml with a range of 72 to 85. There was a trend for the complement activity to increase in all surviving groups after the challenge exposure.

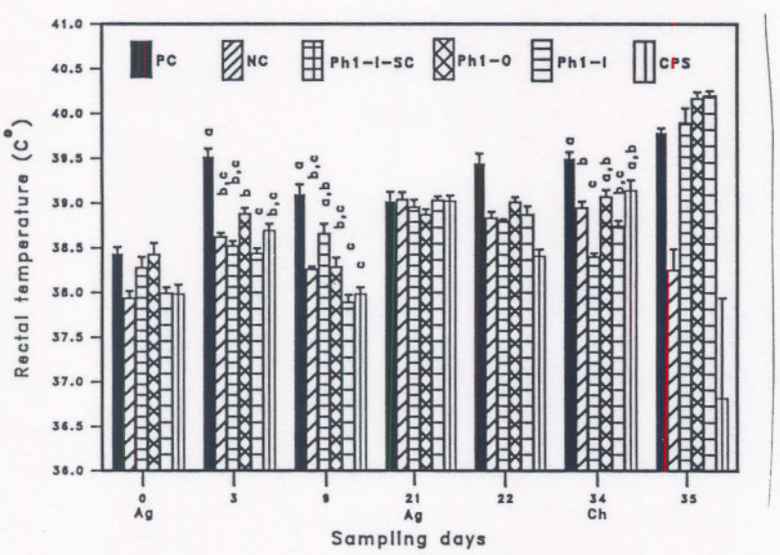

Fig. 2. Mean rectal temperature of goats treated with *Pasteurella haemolytica* A1 (Ph1) vaccines (treatment groups PC = positive controls (live Ph1 in agar beads), NC = negative controls (agar beads only), CPS = Ph1 capsular antigen incorporated inside agar beads, Ph1-O = ultraviolet-inactivated Ph1 whole cell bacterin mixed with agar beads, Ph1-I = ultraviolet-inactivated Ph1 whole cell bacterin incorporated inside agar beads, Ph1-I-SC = ultraviolet-inactivated Ph1 whole cell bacterin incorporated inside agar beads and given subcutaneously). Each goat (except goats in the SC group) was injected into the left lung with appropriate material (Ag) for the group on d 0 and 21. All goats were challenge exposed (Ch) on d 34 and euthanatized (Eu) on d 38. Means with different superscripts are significantly different among treatments ($P<0.05$).

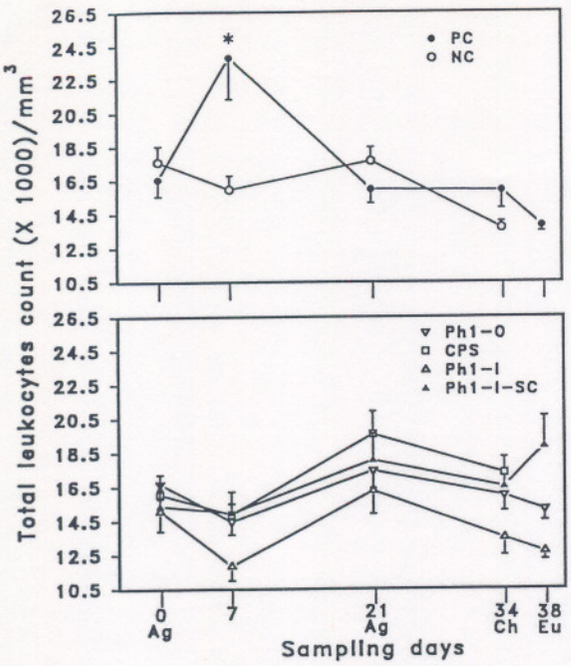

Fig. 3. Mean WBC count/mm^3 of goats treated with *Pasteurella haemolytica* A1 CPS, ultraviolet-killed bacterin, and live Ph1 vaccine. See Fig. 2 for key to abbreviations and methods. Asterisk means significant difference among treatments on day indicated ($P<0.05$).

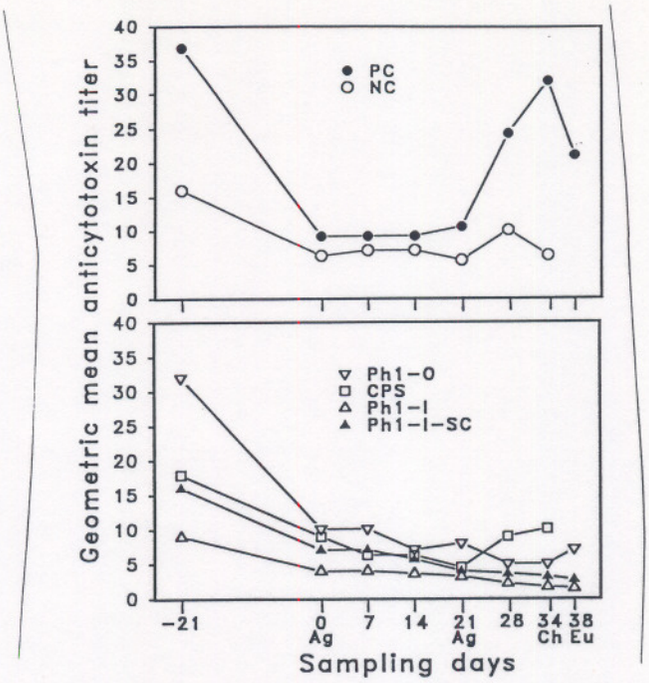

Fig. 4. Anticytotoxin antibody titer (geometric mean) of goats treated with *Pasteurella haemolytica* A1 CPS, ultraviolet killed bacterin, and live Ph1 vaccine. See Fig. 2 for key to abbreviations.

Serum Ph1 IHA antibodies

The geometric mean Ph1 IHA antibody titers (Fig. 6) of all groups were low (< 1:9) on d −21 (< 1:9) and 0 (< 1:2). Seven d after the first antigen injection a primary antibody response occurred in all groups except NC. The response was highest in the PC (1:2048) and the CPS (1:1290) groups. A 5-fold increase in IHA antibody titer occurred in the Ph1-I group after the second antigen injection. The antibody titers of the PC and CPS groups did not change after the second antigen injection and after the challenge exposure. During the same time period, Ph1-I and Ph1-I-SC group mean antibody titers rose. The Ph1-O group had over a 10-fold increase in antibody titer after the challenge exposure. The Ph1 IHA antibody titer of the NC group remained low (< 1:8) on all sampling days.

Pasteurella isolation

Excluding the PC group, no Ph1 isolates (out of 240 attempts) were recovered from the nasal turbinates of the treatment groups prior to the second antigen injection. *Pasteurella haemolytica* A2 isolates were recovered from one NC goat on d 7 and 10, and from one PC goat on d 2, 6, and 10. There were eight Ph1 isolates recovered from three PC goats on d 6, 7, 10, 14, and 21. Also, Ph1 isolates were recovered from the turbinates of two of the three PC goats which died of pneumonic pasteurellosis 2 d after initial inoculation of their lungs with live Ph1. After the second antigen inoculation, and prior to challenge exposure, Ph1 isolates were recovered from one NC goat on d 24 and one PC goat on d 23. One *Pasteurella multocida* isolate (out of 108 attempts) was recovered from a Ph1-I-SC goat on d 24.

After challenge exposure, four Ph1 isolates (out of 20 attempts) were recovered from the nasal turbinates of two PC goats on d 35, and 37. Eight Ph1 isolates (out of 24 attempts) were recovered from the nasal turbinates of three PH1-O goats. Ten Ph1 isolates (out of 20 attempts) were recovered from four goats in the Ph1-I group. Ten Ph1 isolates were recovered (out of 22 attempts) from five goats in the Ph1-I-SC group. Five Ph1 isolates (out of six attempts) were recovered from goats in the NC group. Four Ph1 isolates (out of 11 attempts) were recovered from goats in the CPS group.

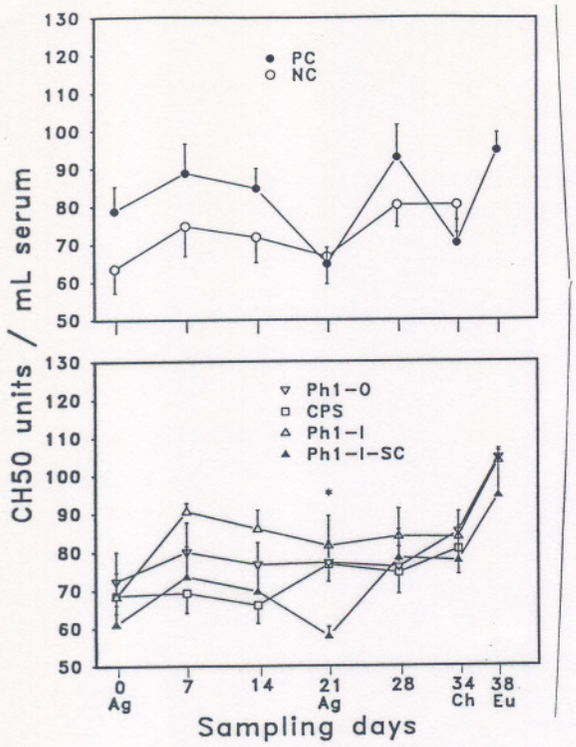

Fig. 5. Mean serum complement activity (CH_{50} units/ml) of goats treated with *Pasteurella haemolytica* A1 CPS, ultraviolet-killed bacterin, and live Ph1 vaccine. See Fig. 2 for key to abbreviations. Asterisk means significance difference among treatments on day indicated ($P<0.05$).

Pasteurella haemolytica A1 isolates were recovered from the left posterior lobe (vaccinated) of goats as follows: NC, 100%; PC, 40%; Ph1-0, 17%; Ph1-I, 0%; Ph1-I-SC, 17%; CPS, 83%. The number of goats per group and mean Ph1 titers (CFU/ml) recovered from the right caudal lobe (challenge exposed) were as follows: five PC, 1.9×10^2; six NC, 2.2×10^8; six Ph1-O, 4.3×10^4; five Ph1-I, 4.4×10^6; six Ph1-I-SC, 4.9×10^6; and six CPS, 8.8×10^5.

Necropsy results

There were significant differences ($P<0.04$) among treatment groups in the mean volume of consolidated lung tissue (Fig. 7). The mean volume of consolidated right lung tissue (cm^3) by treatment groups were as follows: PC group, 0.06; NC group, 150.83; Ph1-O group, 11.68; Ph1-I group, 11.24; Ph1-I-SC group, 33.47; and CPS group, 113.98.

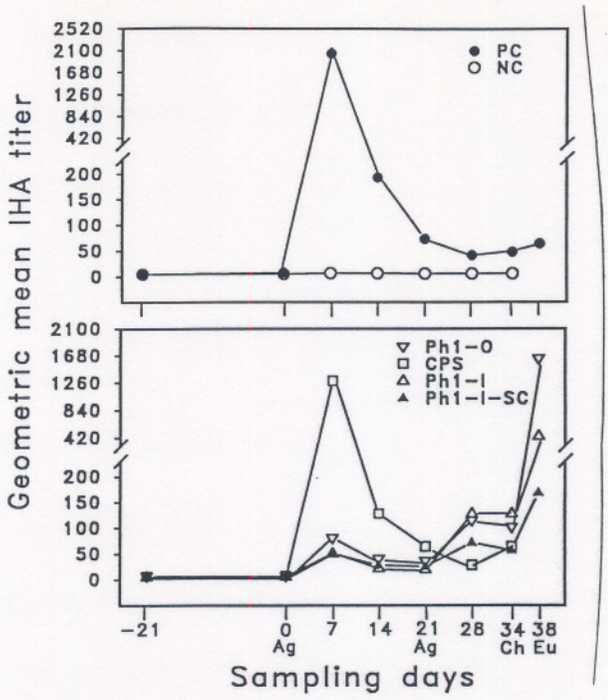

Fig. 6. Indirect hemagglutination antibody titer (geometric mean) of goats treated with *Pasteurella haemolytica* A1 CPS, ultraviolet-killed bacterin, and live Ph1 vaccine. See Fig. 2 for key to abbreviations and methods.

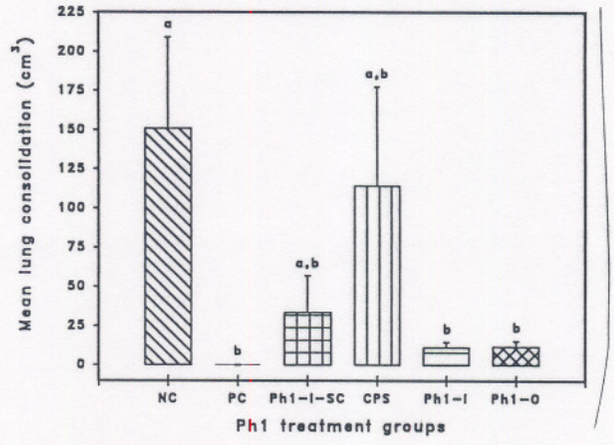

Fig. 7. Mean volume (cm^3) consolidated lung tissue of goats treated with *Pasteurella haemolytica* A1 CPS, ultraviolet-killed bacterin, and live Ph1 vaccine. Means with different superscripts are significantly different among treatments ($P\leq0.05$). See Fig. 2 for key to abbreviations and methods.

4. Discussion

The use of a transthoracic injection of live Ph1 bacteria as a 'vaccine' needs further explanation of why it is used. We call these animals PC. The reason for this group is to establish an immunity which can not be surpassed. It is only an experimental model of the naturally occurring disease. Many diseases have a specific target organ which is most affected during the infection. Usually the best induced immunity that can be obtained is by injecting (immunizing) the target organ (inducing a focal pneumonia), thus mimicking the natural disease. Non-living protective-antigens injected into the target organ are also expected to induce the immunity, but probably not as good as the living bacteria. If this immunity is not obtained then it would be less than reasonable to give them by conventional routes of inoculation. This technique was never intended to be a practical method of vaccination. The immunity induced is our 'gold standard' whereby we compare any experimental vaccine given by the usual (practical) routes of inoculation.

A significant increase in mean rectal temperature (Fig. 2) occurred in the PC group after the goats were injected with virulent live Ph1. This was expected because in previous experiments pneumonia was induced at the injection site (Purdy et al., 1990). This bacterial multiplication at the injection site in the PC group induced solid immunity to a subsequent Ph1 challenge exposure. However, if too many bacteria are injected, the resulting pneumonia may overwhelm the susceptible goats. This occurred in three PC goats 2 d after the initial injection. It is therefore hazardous to use highly virulent bacteria to induce immunity. However, the degree of immunity induced in the survivors by such a procedure is usually of a solid nature and is what we used for comparison.

There was a significant difference (Fig. 3) in the total WBC counts among the treatment groups on d 7, because of the high WBC counts in the PC goats. This might be expected in all studies where log phase bacteria are injected into lung tissue, however significant differences in total WBC counts between other treatment groups occur infrequently in our experience (Purdy et al., 1990, 1993).

The IHA antibody mean titer increased on d 7 in both the PC and the CPS groups, however this increase in the CPS group provided no protection against challenge exposure. Complement activity appeared to increase after the injection of live Ph1 and ultraviolet-killed Ph1 vaccine but not in the CPS or NC groups. The trend for complement activity not to increase after the injection of Ph1 subunit antigens has been reported (Purdy et al., 1993). Serum complement appears to be needed in the removal of live or killed Ph1, but it is not as involved in removing purified Ph1 antigens. Complement in the presence of Ph1 opsoninic antibody rapidly kills Ph1 (Purdy et al., 1989).

All treatment groups had a decrease in anticytotoxic antibody titers between d −21 and 0. This decrease suggested a depleting passive antibody titer. The PC group was the only group to make active anticytotoxin antibody (Fig. 4) after two injections of live Ph1. However, the increase only occurred after the second injection. The absence of anticytotoxin antibody in the Ph1-O, Ph1-I and Ph1-I-SC groups appeared to have no effect on protective immunity against challenge exposure. Purdy et al. (1993) and Conlon and Shewen (1990) have reported that anticytotoxin antibody appears to have little effect on protective immunity against Ph1 challenge exposure. Protective immunity against a severe lung challenge exposure occurred in the PC, Ph1-O, and Ph1-I groups based on the smaller mean volume of consolidated lung tissue (Fig. 7) when compared to the NC and CPS groups. By the same criteria the Ph1-I-SC group was partially protected, however, one Ph1-I-SC goat died due to the challenge exposure. Numbers of surviving goats 4 d after a severe challenge exposure is another determination of protective immunity. All six of the NC goats died on d 35 and five out of six CPS goats died by d 36. These two groups were not protected from the Ph1 challenge exposure, as also indicated by degree of lung consolidation in the survivors.

Ultraviolet irradiation was very effective in killing Ph1 (Lo and MacDonald, 1991). The ultraviolet irradiation leads to the formation of covalent bonds between adjacent pyrimidine residues in the same DNA strand. These pyrimidine dimers inhibit DNA synthesis and thus shut down cell multiplication. It was suggested (Lo and MacDonald, 1991) that Ph1 may lack some of the mechanisms to repair ultraviolet-induced DNA damage. Ultraviolet irradiation has little or no effect on the bacterial cell surface, where the important immunogens appear to be located (Confer et al., 1985). It was reported (Whiteley et al., 1991) that ultraviolet-

killed Ph1 resembled live Ph1 at inducing fibrin exudation, platelet aggregation, and epithelial damage after intratracheal inoculation. However, the inflammation induced by the ultraviolet-killed bacteria was less severe than that induced by the live Ph1.

Rapid clearance of Ph1 from the lungs has been reported by investigators (Lillie and Thomson, 1972; Gilmour et al., 1989, 1990). Agar beads have been successfully used to slow the clearance of *Pseudomonas aeurginosa* (Cash et al., 1979), extracellular products of *Pseudomonas cepacia* (Straus et al., 1988), and *Pasteurella haemolytica* (Purdy et al., 1990). The procedure of incorporating bacteria or antigen in the agar beads has some limitations. For example, if the antigen or bacteria were very heat labile (e.g., destroyed by temperatures greater than 46°C) this technique would not be satisfactory. Washing the oil from the newly formed beads requires preparation time. In this study, the ultraviolet-killed Ph1 bacterin mixed with the beads (Ph1-O group) was as effective as impregnating ultraviolet-killed Ph1 in the beads (Ph1-I group) at producing immunity against Ph1 challenge. Therefore, since beads can be prepared and washed prior to the experiment, there will be no loss of antigen during the repeated washing. This is a potential problem when antigen is incorporated into beads to be washed.

In this study, the ultraviolet-inactivated whole cell Ph1 (inside or mixed with agar beads) was nearly as effective at inducing immunity as the controlled live lung infection. The immunity induced by the subcutaneous route could possibly be improved by injecting a larger Ph1 immunogenic mass incorporated with an appropriate adjuvant.

The CPS antigen was not protective against the transthoracic challenge exposure given in this study. In a previous experiment (Purdy et al., 1993) we appeared to get partial immunity with a similar Ph1 capsule antigen. The reason for the difference is not known, however, different challenge doses and the heterologous challenge used in this study may play a role in the difference.

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More details? Yes